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(54) Title: APTAMERS FOR COMPLEMENT PROTEIN C3b

(57) Abstract

Nucleic acid molecules that specifically bind complement protein C3b are described. The C3b aptamers may be used diagnostically *in vivo* or *in vitro* to detect C3b in a mixture or a biological sample or may be used therapeutically to inhibit undesirable C3b-mediated complement events, for example, to treat inflammation.

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APTAMERS FOR COMPLEMENT PROTEIN C3b

Field of the Invention

This invention is generally in the field of nucleic acid molecules called aptamers that bind complement protein C3b. Such aptamers may be employed therapeutically to inhibit C3b function and thereby to treat complement-mediated disorders. The aptamers may also be employed to detect C3b protein in *in vitro* and *in vivo* applications.

Background of the Invention

The complement system is an important means by which a host defends itself against infection. This system was initially characterized as consisting of a series of approximately twenty serum proteins that can be activated by antibody-antigen complexes or by infecting microorganisms. Complement activation involves a cascade of proteolytic reactions that leads to release of inflammatory mediators and results in the assembly of membrane attack complexes which, in turn, lyse invading microbial cells.

Activation of complement proteins can occur through two pathways: the classical pathway and the alternative pathway (see, Figure 1). One reaction common to both pathways is where the complement protein C3 is cleaved to form the C3a and C3b proteins. C3b is involved in a number of complement-mediated reactions. For example, in the alternative pathway, C3b can associate with the fragment Bb of factor B to form a C3 convertase to produce more C3a and C3b proteins. In the classical pathway, C3b can associate with the C4b and C2a complement proteins to form a C5 convertase to form C5a and C5b complement proteins.

The cleavage products C3a and C5a generated by the convertases of the classical and alternative pathways are also responsible for complement-mediated inflammatory responses typically associated with complement activation at a site of infection. These proteolytic complement fragments promote vasodilation and the attraction and infiltration of phagocytic white blood cells (macrophages, neutrophils) into the sites of infection, where they ingest the infecting microorganisms, resulting in inflammation of the infiltrated tissue. However, despite the clear benefit of an inflammatory response for concentrating phagocytic cells to a local area of infection, an inappropriate complement response can also result in undesirable tissue destruction.

Accordingly, there is a need for means and methods of monitoring and therapeutically modulating complement-mediated events such as inflammation. Agents capable of monitoring complement-mediated events would be useful in detecting early events in phenomena that result from activation of the complement system and in monitoring the course of complement activation. Agents capable of modulating complement-mediated events would be useful for therapeutic intervention, to attenuate the complement cascade and prevent or limit the destruction of tissues that might result from unregulated complement activity. Furthermore, there is a need for diagnostic therapeutic agents that can be easily and efficiently synthesized, using known materials and methodologies.

Summary of the Invention

This invention provides nucleic acid molecules called aptamers that specifically bind the human complement protein C3b, a key protein of the complement system.

The C3b aptamers of this invention include nucleic acid molecules comprising the sequences SEQ ID NO:1 - 45 which bind the C3b complement protein. The C3b aptamers described herein include C3b aptamers that bind to C3b and also inhibit C3b function. C3b function can be routinely assayed using, for example, a standard hemolytic assay. C3b aptamers that bind C3b but do not inhibit certain C3b functions are also described.

In addition, the C3b aptamers described herein also may be modified to form derivatives that have increased stability to nucleases *in vivo* due to the replacement of certain nucleotides or modification of the internucleotide linkages. Such nuclease-resistant derivatives include aptamers having phosphorothioate and/or substituted phosphonate (e.g., methylphosphonate) linkages in place of the usual phosphodiester internucleotide linkages of nucleic acid molecules.

The C3b aptamers of this invention may be used diagnostically to detect C3b in a mixture. Since the C3b aptamers are deoxyribonucleic acid (DNA) sequences, any of a variety of methods for labeling and detecting DNA molecules may be used with C3b aptamers including 5' end-labeling with a radiolabeled nucleotide, incorporation of radiolabeled nucleotides into the aptamer sequence, and biotinylation for non-radioactive labeling systems.

The C3b aptamers of this invention may also be used therapeutically when administered to a person or other vertebrate to inhibit C3b-mediated disorders or phenomena, such as complement-mediated inflammation.

These and other features and advantages of the present invention may be better understood by considering the following detailed description of certain preferred embodiments of the invention.

Brief Description of the Drawings

Figure 1 is a diagram of the complement system of proteins in both the alternative and the classical pathways illustrating the formation and participation of complement protein C3b in both pathways.

Figures 2A and 2B illustrate representative sequences of C3b aptamers according to the invention, grouped into ten families. Family I aptamers have nucleotide sequences comprising SEQ ID NOS:1 - 4. Family II aptamers have nucleotide sequences comprising SEQ ID NOS:5 - 7. Family III aptamers have nucleotide sequences comprising SEQ ID NOS:8 - 14. Family IV aptamers have nucleotide sequences comprising SEQ ID NOS: 15 - 18. Family V aptamers have nucleotide sequences comprising SEQ ID NOS:19 - 22. Family VI aptamers have nucleotide sequences comprising SEQ ID NOS:23 - 37. Family VII aptamers have nucleotide sequences comprising SEQ ID NOS:38 and 39. Family VIII aptamers have nucleotide sequences comprising SEQ ID NO:40. Family IX aptamers have nucleotide sequences comprising SEQ ID NO:41. Family X aptamers have nucleotide sequences comprising SEQ ID NO:42. Hyphens and spaces within sequences are inserted merely to align the sequences to illustrate regions of consensus and do not represent additional nucleotides.

Figure 3 is a bar graph showing percent (%) inhibition of C3-dependent hemolysis of sensitized sheep erythrocytes versus concentration of DNA molecules in a standard hemolysis assay. A C3b aptamer having the nucleotide sequence of SEQ ID NO:6 (solid bar) is compared with a random DNA sequence (hatched bar).

Figure 4 is a bar graph showing percent (%) inhibition of C3-dependent hemolysis of sensitized sheep erythrocytes versus 0.25 mg/ml C3b aptamer molecule having the nucleotide sequence SEQ ID NO:6 (60 mer), versus a derivative C3b aptamer molecule having the nucleotide sequence SEQ ID NO:43 (50 mer), versus a derivative C3b aptamer molecule having the nucleotide sequence SEQ ID NO:44 (40 mer), versus a derivative

molecule having the nucleotide sequence of SEQ ID NO:45 (37 mer), and versus a random DNA control.

Detailed Description of the Invention

In recent years, certain nucleic acid molecules (called aptamers) have been identified that bind specific molecules or compounds (target molecules) such as specific organic dyes, proteins, and other nucleotides (see, e.g., Joyce, *Scientific American*, *267(6)*: 90 - 97 (1992); Bock et al., *Nature*, *355*: 564 - 566 (1992); Wang et al., *Biochemistry*, *32*: 11285 - 11292 (1993); Sassanfar et al., *Nature*, *364*: 550 - 553 (1993)). Typically, aptamers have been isolated from a mixture or library of oligonucleotide molecules (generally less than 100 nucleotides in length) generated using synthetic or recombinant oligonucleotide technology. Such libraries usually consist of approximately 10^{10} to 10^{13} different oligonucleotide molecules (Bock et al., 1992; Sassanfar et al., 1993). The ability to isolate a particular aptamer has depended, *inter alia*, on the ability to generate a mixture or library of nucleic acid molecules of statistically significant complexity; the ability to efficiently distinguish and separate from the library those few members (generally less than 1%) that preferentially bind the target molecule; and the ability to adequately amplify and synthesize those separated aptamers in the amounts necessary to permit purification, further characterization, and therapeutic or other commercial uses.

This invention provides ten families of C3b aptamers which are nucleic acid molecules that specifically bind the complement protein C3b. Such C3b aptamers may be used therapeutically to modulate undesirable complement-mediated disorders or phenomena (such as to inhibit complement-mediated inflammation), they may be used diagnostically to detect C3b *in vivo* or *in vitro*, e.g., in biological samples.

C3b Aptamer Sequences

Forty-two C3b aptamers (SEQ ID NOS:1 - 42) isolated as detailed below were sequenced and grouped into ten families (Families I - X) based on sequence similarity, if any, as shown in Figures 2A and 2B. Four additional DNA sequences (SEQ ID NOS:43 - 46) were derived from the parent C3b aptamer sequence of SEQ ID NO:6 and then examined for C3b binding activity and C3b inhibitory activity (see below).

In another embodiment of this invention, the C3b aptamers contain nucleotide analogs which enhance resistance to nucleases without disrupting the ability of the aptamers to bind and/or inhibit C3b-mediated phenomena. Such nucleotide analogs are known in the

art and include phosphorothioates (see, for example, Putney et al., *Proc. Natl. Acad. Sci. USA, 78*: 7350 - 7354 (1981); Eckstein, *Ann. Rev. Biochem., 54*: 367 - 402 (1985)) and non-ionic alkylphosphonates and arylphosphonates (see, for example, Stec et al., *J. Org. Chem., 50*: 3906 - 3913 (1985); United States Patent 4,511,713).

The DNA molecules described herein can be synthesized using routine DNA synthesis chemistry, such as the solid phase phosphate triester method or the phosphoramidite method (see, e.g., Caruthers, in Synthesis and Applications of DNA and RNA, chapter 3, pages 47 - 94 (Academic Press, Inc., New York, 1987), and, advantageously, by employing an automated DNA synthesizer (e.g., from Applied Biosystems, Foster City, CA; Perkin-Elmer Corp., Foster City, CA; Beckman Instruments, Inc., Fullerton, CA). Aptamers of a specific DNA sequence may also be obtained on order from a commercial vendor of custom nucleic acid molecules (for example, Operon Technology, Inc., Alameda, CA).

The DNA sequences described herein may also be amplified using a standard polymerase chain reaction (PCR) protocol (e.g., GeneAmp PCR Reagent Kit with AmpliTaq™ Polymerase, Catalog No. N801-0055, Perkin-Elmer, Foster City, CA). Because primers are required for the polymerization steps in PCR, appropriate PCR primer binding sites that are complementary to the PCR primers are synthesized and attached to the 5' and 3' ends of a DNA sequence to be amplified using standard recombinant DNA methods employed for performing PCR.

C3b Binding Activity

The ability of the C3b aptamers described herein to bind the C3b protein can be demonstrated and characterized by any of a variety of possible methods including affinity chromatography, surface plasmon resonance (BIAcore™, Pharmacia Biosensor AB, Piscataway, NJ), and standard electrophoretic mobility shift assays (EMSA) (see, e.g., Current Protocols in Molecular Biology, pages 12.2.1 - 12.2.10 (Ausubel et al., eds.) (John Wiley & Sons, New York, NY, 1995). The ability of an aptamer to bind C3b in any of these assays indicates that it may be employed for therapeutic and/or diagnostic applications.

C3b Inhibitory Activity

The ability of a C3b aptamer described herein to inhibit C3b function can be determined by using a standard complement-mediated hemolytic assay. In this assay, sheep erythrocytes are contacted (sensitized) with antibody directed against the erythrocytes. In

the presence of complement proteins, as supplied usually by addition of human serum, the sensitized sheep erythrocytes will be lysed. Lysis of erythrocytes is detected as release of hemoglobin, which can be monitored and quantitated spectrophotometrically. Lysis will be inhibited if a C3b aptamer of this invention inhibits C3b function in the complement cascade (see Figure 1). Thus, the ability to inhibit the complement-mediated hemolytic assay is taken as evidence that a particular C3b aptamer may be useful therapeutically for inhibiting or modulating undesirable complement-mediated disorders or phenomena, including complement-mediated inflammation, which is dependent on the same complement pathways responsible for lysis in the standard complement-mediated hemolytic assay.

Derivative Aptamers

The various C3b aptamer sequences listed in Figures 2 and 3 can be modified to make derivatives with improved features, for example, an enhanced resistance to nucleases, which can increase the half-life of an aptamer *in vivo*; a higher affinity for C3b; and an alteration in the ability of an aptamer to inhibit C3b function where such inhibition is undesirable, for example, in an *in vivo* diagnostic procedure such as magnetic resonance imaging (MRI).

A well known method of enhancing resistance to nucleases is to replace the normal internucleotide phosphodiester linkages with other linkages that do not significantly alter charge or geometry of the nucleic acid. This is commonly accomplished by synthesizing nucleic acid molecules with one or more nucleotide analogs, such as phosphorothioate or phosphonate nucleotide analogs, in place of the usual deoxyribonucleotides in a DNA sequence (see, for example, Eckstein, *Ann. Rev. Biochem.*, *54*: 367 - 402 (1985); Gallo et al., *Nucleic Acids Res.*, *14*: 7405 - 7420 (1986); Stec et al., *J. Org. Chem.*, *50*: 3908 - 3913 (1985); Stec et al., *J. Am. Chem. Soc.*, *106*: 6077 - 6079 (1984)). Such replacements alter the internucleotide phosphate linkage but not the actual sequence of the aptamer.

In addition, if a particular aptamer sequence is found to contain an endonuclease recognition site, a derivative of the aptamer can be synthesized with a single base change to destroy the recognition site. The derivative nucleic acid molecule can then be tested for its ability to bind C3b by a standard method, such as affinity chromatography, surface plasmon resonance using a BIAcore™ system, or electrophoretic mobility shift assay, and for its ability to affect C3b function, such as a standard complement-dependent hemolytic assay as described above (also see, Examples, below).

Another class of derivatives of the aptamers described herein is made by synthesizing deletion derivatives which lack certain portions of the nucleotide sequence of a known C3b aptamer. Such derivatives may, for example, have a smaller size without significant loss of C3b affinity or inhibition activity, which may improve therapeutic administration. Alternatively, a deletion derivative may have an altered C3b affinity and/or C3b inhibition activity which is advantageous for a particular application.

To make deletion derivatives of a particular C3b aptamers described herein, it is generally useful to use a computer program, such as MacDNASIS® Version 3.0 (Hitachi Software Engineering America, Ltd., San Bruno, CA) to generate a secondary structure of the known C3b aptamer and then, depending on the desired result, to selectively delete (by design) regions that may or may not be intimately involved in the secondary structure. For example, a computer-generated secondary structure for the C3b aptamer having the sequence of SEQ ID NO:6 indicated that the 3' terminal ten nucleotides were not involved in intrastrand hydrogen bonding in the secondary structure. A set of deletion derivatives was then designed and synthesized from this parent C3b aptamer to eliminate selected portions of the aptamer sequence.

The parent C3b aptamer has a 60 nucleotide sequence (60 mer) of SEQ ID NO:6 and was shown to bind C3b with a significantly higher affinity than six other representative aptamer sequences (SEQ ID NOS:1, 8, 15, 19, 26, and 41). The dissociation constant (Kd) for the 60 mer having the sequence of SEQ ID NO:6 was calculated from BIAcore™ system measurements to be approximately 5×10^{-6} M. Furthermore, in the C3-dependent hemolytic assay used to measure the ability of a DNA molecule to inhibit C3b function, the same 60 mer C3b aptamer inhibited hemolysis 100 % at a concentration of 0.25 mg/ml.

Deletion derivative sequences of SEQ ID NO:6 were synthesized and compared for their ability to bind C3b and to inhibit C3b function as determined using the complement-dependent hemolytic assay. A 50 nucleotide (50 mer) derivative was synthesized having a nucleotide sequence of SEQ ID NO:43 which lacks the 3' terminal ten nucleotides not involved in intrastrand hydrogen bonding in the computer generated secondary structure of the parent C3b aptamer. This 50 mer bound C3b with approximately the same affinity as the parent 60 mer (SEQ ID NO:6) sequence, but inhibited hemolysis by approximately 55 % (see Figure 4). Accordingly, the removal of ten nucleotides from the 3' end of SEQ ID NO:6 does not appear to significantly affect C3b binding but does result in a diminished ability to inhibit C3b function. A 37 mer derivative (SEQ ID NO:45) retained

approximately the same affinity as the parent 60 mer (SEQ ID NO:6) for C3b, but lost the ability to significantly inhibit C3b function since the 37 mer inhibited the hemolysis assay to about the same extent as a DNA molecule having a random sequence, i.e., 8 - 10 % (see, Figure 4).

A 40 mer deletion derivative sequence (SEQ ID NO:44) exhibited a 30-fold higher affinity for C3b than the parent 60 mer C3b aptamer (SEQ ID NO:6) with a Kd of 1.5 x 10^{-7} M. Surprisingly, this 40 mer sequence only inhibited the hemolysis assay to about the same extent as a DNA molecule having a random sequence (8 - 10 %). Thus, an aptamer having the sequence of SEQ ID NO:44 binds C3b but does not significantly inhibit C3b function in the hemolysis assay. (The aptamer may inhibit some other C3b function.) Such features suggest that the 40 mer (SEQ ID NO:44) may be particularly well suited for *in vitro* (e.g., in a microtiter plate assay system) or *in vivo* (e.g., MRI) diagnostic applications where detection of C3b is the primary goal and inhibition of C3b function in the complement system is either not important or not desired.

C3b Aptamers in Therapeutic Use and Pharmaceutical Compositions

C3b aptamers can be used therapeutically to inhibit undesirable complement-mediated disorders or phenomena, such as complement-mediated inflammation, which, if unchecked, may cause damage to, or necrosis of, tissue.

The efficacy of a therapeutic use of a C3b aptamer of this invention will depend, in part, on an aptamer's half-life *in vivo*, which is most likely to be limited by various *in vivo* nuclease activities. It is generally known that resistance to nuclease degradation may be enhanced by utilizing one or more possible strategies, including use of liposome-based delivery systems, by frequent (e.g., daily) administration of the nucleic acid molecule, by use of time-released delivery systems, and by modification of internucleotide phosphodiester linkages. Particular mention is made of the use of phosphonate and phosphorothioate nucleotide analogs, which improve the resistance of DNA molecules to degradation but do not significantly alter the structural geometry of DNA molecules so that aptamer binding and functional activities are not significantly altered (see, for example, Eckstein, *Ann. Rev. Biochem.*, *54*: 367 - 402 (1983); Gallo et al., *Nucleic Acids Res.*, *14*: 7405 - 7420 (1986); Stec et al., *J. Org. Chem.*, *50*: 3908 - 3913 (1985); Stec et al., *J. Am. Chem. Soc.*, *106*: 6077 - 6079 (1984); Zhu et al., *Science*, *261*: 209 - 211 (1993)).

Depending on the intended mode of administration, a C3b aptamer according to the invention can be advantageously formulated as a pharmaceutical composition, which can

take the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, or the like, preferably in unit dosage form suitable for single administration of a precise dosage. Such compositions will include an effective amount of the C3b aptamer, optionally in combination with a pharmaceutically acceptable carrier, and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, diluents, etc. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, that is, the material may be administered to an individual along with the C3b aptamer without either causing any undesirable biological effect or interacting in a deleterious manner with the structure and function of the C3b aptamer or of any other component that may be incorporated into the pharmaceutical composition.

C3b aptamers can be used directly or in combination with an appropriate pharmaceutical carrier to form a pharmaceutical composition for treating undesirable complement-mediated phenomena, especially complement-mediated inflammation. Examples of typical pharmaceutical carriers, used alone or in combination, include one or more solid, semi-solid, or liquid diluents, fillers and formulation adjuvants which are non-toxic, inert and pharmaceutically acceptable. Such pharmaceutical compositions are preferable in dosage unit form, that is, physically discrete units containing a predetermined amount of the drug corresponding to a fraction or multiple of the dose which is calculated to produce the desired therapeutic response, conventionally prepared as tablets, lozenges, capsules, powders, aqueous or oily suspensions, syrups, elixirs, and aqueous solutions.

For solid compositions, conventional non-toxic, pharmaceutically acceptable carriers may include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving or dispersing an active compound as described herein and optimal pharmaceutical adjuvants in an excipient, such as, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, triethanolamine oleate, etc.

Recently, a delivery system was developed in which nucleic acid is encapsulated in cationic liposomes which can be injected intravenously into a mammal. This system has

been used to introduce DNA into the cells of multiple tissues of adult mice, including endothelium and bone marrow (see, for example, Zhu et al., *Science*, *261*, pages 209-211 (1993); incorporated herein by reference). Such liposome-based delivery systems for nucleic acid molecules are applicable to the C3b aptamers of this invention.

The C3b aptamers may be administered orally, parenterally (for example, intravenously), by intramuscular injection, by intraperitoneal injection, or by other routes that permit the C3b aptamer to come in contact with the proteins of the complement system to exert their therapeutic effect, for example, to inhibit complement-mediated inflammation. The exact amount of C3b aptamer required will vary from subject to subject, depending on the age, weight and general condition of the subject, the severity of the disease that is being treated, the state of complement activation, the location and size of the infection, the particular aptamer used, its mode of administration, and the like. Thus, it is not possible or necessary to specify an exact effective amount. Appropriate amounts for therapeutic use may be routinely determined by skilled practitioners using only routine experimentation given the teachings herein.

Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art, as are standard basic and clinical pharmokinetic procedures; for example, see Remington: The Science and Practice of Pharmacy, 19th edition, Vols. I & II (Gennaro, A.R. et al., editors) (Mack Publishing Co., Easton, PA, 1995).

C3b Aptamers in Diagnostic Use

As reagents that specifically bind C3b, the C3b aptamers of this invention may also be used diagnostically to detect C3b in mixtures and biological samples *in vitro* or *in vivo*. If a diagnostic scheme depends on labeling and detecting the aptamer bound to C3b, then methods for labeling and detecting nucleic acid molecules are also generally applicable to C3b aptamer. For example, standard methods of labeling DNA molecules with radioactive or non-radioactive labels (such as biotin), including 5' or 3' end-labeling methods and use of polymerase chain reaction (PCR) are well known in the art and appropriate reagents and instructions are commercially available (see, for example, Current Protocols in Molecular Biology (Ausubel et al., eds.) (John Wiley & Sons, New York, 1995); PCR Radioactive Labeling System, Catalog No. 10199-016, PhotoGene™ System and PCR Nonradioactive Labeling Systems, Catalog No. 10200-012, Life Technologies, Gaithersburg, MD; T4 polynucleotide kinase and [γ-^{32}P]ATP system, Catalog No. U2010 and 3' end terminal deoxynucleotidyl transferase and ^{32}P-deoxynucleotides or [α -^{32}P] cordycepin-5'

triphosphate system, Catalog No. U2000, Promega, Madison, WI). C3b aptamers that bind C3b, but do not inhibit C3b function, may be especially well suited for *in vivo* diagnostic procedures such as MRI. An example of such a C3b aptamer is one having the nucleotide sequence of SEQ ID NO:44 (see *infra*). It will be understood by those skilled in the art that the particular labeling system used must not significantly alter the ability of the aptamer to bind C3b.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes and are not to be construed as limiting this invention in any manner.

EXAMPLES

Example 1. DNA Sequences of C3b Aptamers

A nucleotide library of 96-mer polynucleotides was prepared by Operon Technologies (Alameda, CA) having approximately 10^{16} molecules each consisting of a randomized 60-mer flanked by a 5' terminal 18-nucleotide sequence CTGCAGGTCGACGCTAGC (SEQ ID NO:47) and a 3' terminal 18-nucleotide sequence CACGTGGAGCTCGGATCC (SEQ ID NO:48). Part of this library was amplified using polymerase chain reaction (PCR) until a pool of approximately 10^{13} oligonucleotides was obtained. The common 5' and 3' terminal 18-nucleotide sequences provided binding sites necessary for amplifying the oligonucleotides by PCR. Thus, each DNA molecule was a template strand of DNA that could be amplified by PCR. The "3' primer" used in PCR to synthesize a complementary nucleic acid strand (non-template strand) from the template strand had the 5' to 3' sequence ACTATAGGGATCCGAGCTCCACGTG (SEQ ID NO:49) which is partially complementary to the 3' terminal 18-nucleotide sequence (SEQ ID NO:48). The "5' primer" used in the PCR had the same sequence as the 5' flanking sequence SEQ ID NO:47 because the 5' PCR primer was used to synthesize more template strands from non-template strands. Both PCR primers were designed to contain restriction sites that could be used in cloning the aptamers in plasmid vectors. In the experiments described below, aptamers tested for C3b binding ability using C3b-ConA-agarose affinity column chromatography contained 5' and 3' flanking PCR binding sites, whereas aptamers used in the BIAcore™ system to test C3b binding and in the C3-dependent hemolysis assay to test C3b inhibition activity did not have the PCR binding sites. The C3b binding ability

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and the ability to inhibit C3b function were determined for the sequences of SEQ ID NOS: 43 - 46 without the flanking PCR binding sites.

Example 2. Aptamer Binding to C3b-ConA-Agarose Affinity Column

C3b was prepared by tryptic cleavage of purified C3 protein (Sigma Chemical Co., St. Louis, MO) (see, Bokisch et al., *J.Exp.Med.*, *129*:1109-1130 (1969); Tack et al., *Meth.Enzymol.*, *80*:64-101 (1981). C3 (0.666 mg) was incubated with 0.013 mg trypsin phosphate buffered saline for 15 minutes at room temperature to cleave C3 into complement cascade proteins C3a and C3b (both monomers and dimers of C3b are formed) (see Figure 1). The proteolytic cleavage reaction was stopped by addition of 0.3 mg soybean trypsin inhibitor (Sigma). Cleavage products were run on a Bio-Sil® SEC 400 HPLC column (serial no. 234607, BIO-RAD, Richmond, CA) to separate C3b cleavage product from uncleaved C3 and C3b dimers and C3a. C3b was quantified by absorbance at 280 nm and purity assessed by standard SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The purified C3b was assayed by binding to sCR1, a soluble form of the complement receptor type 1 molecule CR1 (see, Weisman et al., *Science, 249*: 146 - 151 (1990)) using a standard ELISA protocol. In this assay, the wells of 96-well microtiter plates were first coated with various amounts (e.g., 0, 1, 3, 5, or 10 μg) of C3b by filling the wells with a solution containing the particular amount of C3b in phosphate buffered saline (PBS, GIBCO BRL, Gaithersburg, MD) supplemented with Tween-20 (5 %) and allowing the C3b to bind to the surface of the wells over the course of 2 hours at room temperature. The supernatant solution was aspirated off and sCR1 added at various amounts ranging from 100 to 1000 ng. The plates were incubated for 90 minutes at room temperature and then washed three times with PBS. The sCR1 bound to the C3b in a well was detected spectrophotometrically at an optical density of 450 nm using horseradish peroxidase-labeled rabbit anti-CR1 polyclonal serum (see, Makrides et al., *J. Biol. Chem.*, *267*: 24754 - 24761 (1992)) and the peroxidase substrate TMB (catalog no. 50-76-00, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Maryland).

Alternatively, the purified C3b was incubated with Factor I and sCR1 which degrade C3b into the characteristic degradation products iC3b and C3dg, which can be identified by electrophoresis on SDS-polyacrylamide gels (Weisman et al., *Science, 249*: 146 - 151 (1990)).

Purified C3b was bound to a concanavalin A (ConA)-agarose resin to form a C3b-Con A-agarose affinity chromatography resin. To prepare the affinity resin, 1 ml of ConA-agarose (Vector Laboratories, Inc., Burlingham, CA) was washed four times with 10 ml S buffer (20 mM Tris-acetate, pH 7.4; 140 mM NaCl; 5 mM KCl; 1 mM $CaCl_2$; 1 mM $MgCl_2$), and resuspended in 10 ml S buffer with 250-500 μg purified C3b. This mixture was incubated overnight on a shaker at 4°C. The affinity resin was recovered by centrifugation, resuspended in 2 ml S buffer and poured into a 0.8 x 4 cm polypropylene column.

The column was washed with 8 ml S buffer. Supernatant from the overnight incubation as well as washes were reserved to assay for unbound C3b.

DNA molecules comprising aptamer sequences were designed to contain PCR primer binding sites at their 5' (SEQ ID NO:47) and 3' (SEQ ID NO:48) ends so that prior to applying to the C3b-Con A-agarose affinity column, the aptamer sequences could be radiolabeled using available PCR primers (e.g., SEQ ID NOS:47 and 49) and a standard PCR labeling protocol (e.g., PCR Radioactive Labeling System, Catalog No. 10199-016, Life Technologies, Gaithersburg, MD). Radiolabeled, single-stranded DNA molecules containing aptamer sequences in S buffer were run through a ConA-agarose column (without C3b) in order to eliminate molecules that bound to the resin. One ml of ConA-agarose was washed with 10 ml S buffer and used to prepare a column. Flow-through material was collected. The column was washed three times with 0.5 ml S buffer and the washes pooled. The wash fractions were reapplied twice to the column and the flow-through collected. Then, the column was washed six times with 0.5 ml S buffer and these washes collected as separate fractions. All fractions were counted and those fractions with highest counts were reserved.

The DNA molecules that did not bind on the ConA-agarose column above were applied to the C3b-ConA-agarose affinity column described above. The flow-through material was collected and reapplied twice. The column was then capped, reloaded and incubated at room temperature for 1 hour. After incubation, the column was uncapped and the flow-through fraction collected. The column was then washed ten times with 0.5 ml S buffer and the washes collected as separate fractions. The column was eluted six times with 0.5 ml 0.1 M α-methyl-D-mannoside (α-MM) in S buffer, which eluted DNA molecules containing aptamer sequences bound to C3b from the affinity column. The

eluates were also collected as separate fractions. DNA in all fractions was measured by Cerenkov counting (counts per minute, cpm) for ^{32}P-labeled DNA.

Eluate fractions, column supernatant, and wash material were assayed for C3b protein using the Bradford method (BioRad, Hercules, CA) and bovine serum albumin (BSA) as a standard. For this assay, samples were diluted as necessary in α-MM. A portion (160 µl) of each sample or various amounts of the BSA protein standard were dispensed into wells of a microtiter dish and 40 µl of undiluted Bradford dye reagent was added to each well. The plates were incubated at least 10 minutes at room temperature and then read spectrophotometrically at 560 to 650 nm as recommended by the manufacturer.

Fractions eluted from the affinity column that contained both DNA (by cpm) and protein (by Bradford assay) were pooled (generally eluate fractions 2, 3, 4). The pooled material was extracted twice with phenol:chloroform. DNA was precipitated by the addition of 0.1 volume 3 M sodium acetate, 2 - 2.5 volumes ice cold ethanol and glycogen as carrier. Precipitates were incubated at -20°C for >2 hours, then centrifuged for 15 min. at 4°C in an Eppendorf microcentrifuge. Pellets were washed with 500 µl ice cold 70% ethanol and 5 min. at 4°C. Supernatants were reserved and counted to detect ^{32}P. Tubes were covered with perforated parafilm and dried 15 - 20 min. in a Speed-vac (Savant). DNA pellets were resuspended in a total volume of 100 µl distilled H_2O and stored at -20°C.

Example 3. Cloning and Sequencing of Aptamers

To amplify and sequence aptamers, single-stranded DNA molecules containing aptamer sequences were first made double-stranded using standard PCR (GeneAmp PCR Reagent Kit with AmpliTaq™ Polymerase, catalog no. N801-0055, Perkin Elmer-Cetus, Foster City, CA) and the PCR primers (SEQ ID NOS:47 and 49) described above. The double-stranded molecules were then cloned using standard methods (TA Cloning Kit, catalog no. K2000-01, Invitrogen Corp., San Diego, CA). Mini-prep DNA was prepared from the clones for sequencing (QIAprep 8 plasmid kit, catalog no. 17122, QIAGEN, Inc., Chatsworth, CA). Sequencing reactions were performed on the mini-prep DNA using Sequenase Version 2.0 DNA Sequencing kit (catalog no. 70770, United States Biochemical Corp., Cleveland, OH) and commercially available sequencing primers (Sp6 promoter primer, catalog no. N550-02; T7 promoter primer, catalog no. N560-02; Invitrogen Corp., San Diego, CA). Sequencing of the collected C3b-binding molecules revealed forty-two

separate aptamer sequences, which are illustrated in Figures 2A and 2B, grouped in ten structurally similar families, and which are also set forth in the Sequence Listing, *infra* (see, SEQ ID NOS: 1 - 42).

Example 4. Aptamer Binding to C3b in Electrophoretic Mobility Shift Assay (EMSA)

In addition to evaluating aptamer binding to C3b using C3b-ConA-agarose affinity columns, aptamer-C3b complexes were also detected by electrophoretic mobility shift assay (EMSA). In this experiment, radiolabeled aptamer DNA (approximately 3 - 5 x 10^5 cpm) was incubated with 5 - 40 pmol C3b in S buffer for 15 minutes at room temperature in a reaction volume of 30 µl. Five µl of 6X loading buffer (40% glycerol, 0.1% bromophenol blue, 0.1% xylene cyanol) was then added and samples loaded onto a 6% native polyacrylamide gel. After running the gel for approximately 2.5 hours at 200 V, the gel was wrapped in cellophane and exposed directly to X-ray film. C3b-aptamer complexes were detected as material running at a slower mobility than unbound aptamer DNA.

Example 5. Aptamer Binding to C3b Evaluated by Surface Plasmon Resonance (SPR)

The C3b binding ability of seven representative DNA molecules of the 42 aptamers in Figures 2A and 2B having nucleotide sequences of SEQ ID NOS:1, 6, 8, 15, 19, 26, and 41, respectively, were further investigated by surface plasmon resonance (SPR) as determined using the BIAcore™ system according to the manufacturer's recommendations (Pharmacia Biosensor AB, Piscataway, NJ). Of these seven, the C3b aptamer having the 60 nucleotide (60 mer) sequence of SEQ ID NO:6 exhibited a significantly stronger affinity for C3b in the BIAcore™ system than the other aptamers. The data indicated that this 60 mer C3b aptamer (SEQ ID NO:6) bound C3b with a dissociation constant (Kd) of 5 x 10^{-6} M.

Four shorter derivatives of the 60 mer having the sequence SEQ ID NO:6 were synthesized and their ability to bind C3b and to inhibit C3b function determined. SEQ ID NO:43 (AGCCATGACC CAGTAGACTA TGACCGATTC CCCGGAGACC TTCCTGAGCC) is 50 nucleotides in length (50 mer), SEQ ID NO:44 (CAGTAGACTA TGACCGATTC CCCGGAGACC TTCCTGAGCC) is 40 nucleotides in length (40 mer), SEQ ID NO:45 (GACCCAGTAG ACTATGACCG ATTCCCCGGA GACCTTC) is 37 nucleotides in length (37 mer), and SEQ ID NO:46 (AGTAGACTAT GACCGATTCC CCGGAGACCT T) is 31 nucleotides in length (31 mer).

A measure of the C3b binding ability using the BIAcore™ system indicated that whereas SEQ ID NO:43 (50 mer) and SEQ ID NO:45 (37 mer) retained the C3b binding affinity of the parent SEQ ID NO:6 (60 mer), the 31 mer (SEQ ID NO:46) had completely lost the distinctive affinity of the parent SEQ ID NO:6. A 40 nucleotide derivative (40 mer) having SEQ ID NO:44 exhibited a 30-fold increase in C3b binding affinity (Kd of 1.5 x 10^{-7} M) over that of the parent 60 mer (SEQ ID NO:6) C3b aptamer.

Example 6. Hemolytic Assay to Determine C3b Aptamer Inhibition Activity

A complement protein C3-dependent hemolytic assay was used to measure the ability of a C3b aptamer, or a derivative thereof, to inhibit C3b function. Human C3-depleted serum, purified human C3 and antibody-sensitized sheep erythrocytes were obtained from Advanced Research Technologies (San Diego, CA). For the assay antibody-sensitized sheep erythrocytes were lysed using, as a source of complement, C3-depleted human serum which was reconstituted with purified C3 to yield 65.70 % lysis of the erythrocytes. Single-stranded aptamers, or derivatives thereof, were diluted in buffer (20 mM Tris, pH 7.4; 140 mM NaCl; 5 mM KCl; 1 mM $CaCl_2$; 1 mM $MgCl_2$) and preincubated with C3 for 10 minutes at room temperature. The diluted aptamer, or derivative thereof, was then incubated with C3-depleted serum (1.5 %) and sensitized sheep erythrocytes (approximately 1 x 10^7 cells/ml) for 30 minutes at 37°C in V-bottom microtiter plates. The plates were then centrifuged to form cell pellets (1500 rpm, 10 minutes) and the supernatants were transferred to flat bottom microtiter plates. The absorbance at 405 nm was measured spectrophotometrically to quantitate release of hemoglobin as a measurement of cell lysis. Control samples included: a random nucleotide sequence; a sample lacking C3 (to measure C3-independent background lysis); a sample lacking C3 and C3-depleted serum (to measure complement-independent background lysis); and erythrocytes lysed with water (maximum lysis). The parent C3b aptamer having the 60-nucleotide sequence (60 mer) of SEQ ID NO:6 and its deletion derivatives SEQ ID NOS:43 - 45 were assayed for the ability to inhibit C3-dependent lysis of the antibody-sensitized sheep erythrocytes. The results are shown in Figures 3 and 4.

Figure 3 shows the percent inhibition of hemolysis versus the concentration of the parent 60 mer C3b aptamer (SEQ ID NO:6) compared to a DNA molecule having a random nucleotide sequence in the assay. The ability of the 60 mer C3b aptamer was clearly concentration-dependent and significantly better than the DNA molecule of random

nucleotide sequence. The IH_{50} (the concentration of the 60 mer C3b aptamer at which 50 percent of the cells are lysed) was calculated to be 0.09 mg/ml (4.5×10^{-6} M), which corresponds well with its Kd of 5×10^{-6} M (see above).

Figure 4 shows the percent inhibition of hemolysis versus the parent 60 mer C3b aptamer and deletion derivatives of the 60 mer DNA sequence. The 60 mer C3b aptamer inhibited hemolysis 100 % at the concentration used (0.25 mg/ml) and the 50 mer deletion derivative molecule (SEQ ID NO:43) inhibited hemolysis at 55 %. The 40 mer (SEQ ID NO:44) and the 37 mer (SEQ ID NO:45) deletion derivatives inhibited hemolysis to about the same extent as a DNA molecule having a random sequence, 8 - 10 %. This result was surprising for the 40 mer (SEQ ID NO:44) since BIAcore™ system measurements (see above) indicated that the 40 mer actually binds C3b with a 30-fold higher affinity than the parent 60 mer (SEQ ID NO:6). This result indicates that the sequence of the 40 mer (SEQ ID NO:44) permits enhanced binding but does not significantly interfere with C3b function.

Although a number of embodiments and features have been described above, it will be understood by those skilled in the art that modifications and variations of the described embodiments and features may be made without departing from either the spirit of the invention or the scope of the appended claims. The publications cited herein are incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
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(i)APPLICANT:Marsh, Jr., Henry C.,
(i)APPLICANT:Stewart, Sue E.
(ii)TITLE OF INVENTION: APTAMERS FOR COMPLEMENT PROTEIN C3b
(iii)NUMBER OF SEQUENCES:49
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(C)CITY: Boston
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(F)ZIP:02109
(v)COMPUTER READABLE FORM:
(A)MEDIUM TYPE:Floppy disk
(B)COMPUTER:IBM PC compatible
(C)OPERATING SYSTEM:PC-DOS/MS-DOS
(D)SOFTWARE:Word 6.0
(vi)CURRENT APPLICATION DATA:
(A)APPLICATION NUMBER:not yet assigned
(B)FILING DATE: 28 APRIL 1997
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(A)APPLICATION: 08/646,174
(B)FILING DATE: 7 MAY 1997
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(B)REGISTRATION NUMBER:30,237
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(ix)TELECOMMUNICATION INFORMATION:
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(B)TELEFAX:617-345-9111

(2) INFORMATION FOR SEQ ID NO:1:
(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH:60 base pairs
(B)TYPE:nucleic acid
(C)STRANDEDNESS:single
(D)TOPOLOGY:linear
(ii)MOLECULE TYPE:DNA
(xi)SEQUENCE DESCRIPTION:SEQ ID NO:1:
AGCCCAGGAC TCTGCTGATG ACTTAGATAC CCTCCCGGTC CTGTGGCGGA 50

GGACCACCCC 60

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

AGCCCAGGAC TCTGCTGATG ACTTAGATAC CTCCCGGTCC TGTGGCGGAG 50

GACCAGCCCC 60

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:59 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

AGCCCACACT CTGCGATGAC TTAGATACCT CCCGGTCCTA TGGCGGAGGA 50

CCCACCCCC 59

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

AGCCCAGGAC TATAGCTTGA TGATGACTTA GATCCCTCCG GTCCTGTGGC 50

GGAGGACACC 60

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

CAGGCAGAAG CAGAAGACTT TGACCTGACT GACTAAGCTC AGCACTGGCT 50

TAGGTACCCC 60

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

AGCCATGACC CAGTAGACTA TGACCGATTC CCCGGAGACC TTCCTGAGCC 50

CTGAACTCCC 60

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

AGCCATGACC CAGTAGACTA TGACCGATTC CCCCGGAGAC CTTCCTGAGC 50

CTGAACTCCC 60

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

ACAGCAAGAC GCGTTGACTA AGACTTTTGA GCAAATGCTG CCAGTAACAA 50

CGCCGGTTCC 60

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:9:

ACAGCAAGAC GCAGTTGACT AAGACTTTTG AGCAAATGCT GCCAGTAACA 50

ACGCCGGTCC 60

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:10:

ACAGCAAGAC GCACTTGACT AAGACTTTTG AGCAAATGCT GCCAGTAACA 50

ACGCCGGTCC 60

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:11:

CCAGCAAGAC GCAGTTGACT AAGACTTTTG AGCAAATGCT GCCAGTAACA 50

ACGCCGGTCC 60

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

ACAGCAAGAA GGCAGTTGAC TAAGACTTTT GAGCAAATGC TGCCAGTAAC 50

AACGCCGGAC 60

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:61 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

ACAGCAAGAC GCAGTTGACT AAGACTTTTT GAGCACCGGC TGCCAGTAAC 50

AACGCCGGTC C 61

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

CAAGACGCAG TTGACTATGA ACCCCTACAC CGGCTGGAAG ACGTAATGCC 50

TGGGTACCCC 60

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:15:

AGCGAGACAC AGATGACTTA GAACCCACAA CGCGACTGTT ACTCGCCATA 50

TGCCGTGTCC 60

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CAGCGAGACA CAGATGACTT AGAACCCACA ACGCGACTGT ACTCGCCATA 50

TGACCGTTCC 60

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CAGCAGACAC AGATGACTTA GAACCCCACA ACGCGACTGT ACTCGCCATA 50

TGCACGTTCC 60

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGCAGCAGGT CGCAGATGAC TTAGAACTAA CTTAAACACT CGTTTAGCTA 50

GAGCGGATCC 60

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ACACTCAGTG TCCGGGGCTC GGGAAAGCAA GGACTTCAGC TACGCGGCCG 50

CAGTCTGCCC 60

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:20:

ACACTCAGTG TCCGGGGCTC GGGAAAGCAA GGACTTCAGC TACGCGCGAC 50

GCAGTCTGCC 60

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:21:

ACACTCAGTG TCCGGGGCTC GGGAAAGCAA GGACTTCAGC TACGCCCGCC 50

GCAGTCTGCC 60

(2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:22:

ACACTCAGTG TCCGGGGGCT CGGGAAAGCA AGGACTTCAC GTACGCGGCC 50

GCAGTCTGCC 60

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:23:

ACACCAGAGG GTTATGCAGT TGACTGCCGT CGCCATTGTT CTAGGGACGC 50

CTTCGCGTGC 60

(2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:59 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:24:

ACACCAGAGG GTTATGCAGT TGACTGCCGT CGCCATTGTT CTAGGGCGGC 50

CTTCGGCTC 59

(2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:25:

ACACCAGAGG GTTATGCAGT TGACTGCCGT CGCCATTGTT CTAGGACGCC 50

TTCGGGCCTC 60

(2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:26:

ACACCAGAGG TTATGCAGTT GACTGCCGTC GCCATTGTTC TAGGACCGAC 50

TTCGGCAGTC 60

(2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:27:

ACACCAGAGG TTATGCAGTT GACTGCCGTC GCCATTGTTC TAGGACCCGC 50

TTCGGCGCTC 60

(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:28:

ACACCAGAGG TTATGCACTT GACTGCCGTC GCCATTGTTC TAGGACCCGC 50

TTCGGCGCTC 60

(2) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:29:

ACACCAGAGG TTATGCAGTT GACTGCCGTC GCCATTGTTC TAGGACCTGC 50

TTCGGCGCTC 60

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:61 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:30:

ACACCAGAGG TTATCCAGTT GACTGCCGTC GCCATTGTTT CTAGGACCCG 50

CTTCGCGCCT C 61

(2) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:31:

ACACCAGAGG TTATGCAGTT GACTGCCGTC GCCATTGTTC TAGGACCCGC

TTCGGCCTCC

(2) INFORMATION FOR SEQ ID NO:32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:32:

ACACCAGAGG TTTATGCAGT TGACTGCCGT CGCCATTGTT CTAGGACCCG 50

CTTCGGCCTC 60

(2) INFORMATION FOR SEQ ID NO:33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:33:

ACACCAGAGG TTATGCAGTT GACTGCCGTC GCCATTGTTC TAGGACCCGG 50

CTTCGGCCTC 60

(2) INFORMATION FOR SEQ ID NO:34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:34:

ACACCAGAGG TTATGCAGTT ACTGCCGTCG CCATTGTTCT AGGACCCGCA 50

TTCGGCGCTC 60

(2) INFORMATION FOR SEQ ID NO:35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:35:

ACACCAGAGG TTATGCAGTT ACTGCCGTCG CCATTGTTCT AGGACCCGCT 50

TCGGGATTCC 60

(2) INFORMATION FOR SEQ ID NO:36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:36:

ACACCAGAGG TTATGCAGTT ACTGCCGTCG CCATTGTTCT AGGACCCCGC 50

TGCGGGCCTC 60

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ACACCAGAGG TTATGCAGTT ACTGCCGTCG GCCATTGTTC TAGGACCCGC 50

TTCGGCGCTC 60

(2) INFORMATION FOR SEQ ID NO:38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AGCTGGGACC GCAGTTGAAT ATAGACCATA AGGCAAAAGC GACTGAATAA 50

GTTTCCAGCC 60

(2) INFORMATION FOR SEQ ID NO:39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AGCAGGGACC GCAGTTGAAT ATAGACCATA AGGCAAAAGC GACTGAATAA 50

GTTTCCAGCC 60

(2) INFORMATION FOR SEQ ID NO:40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

AGCAAGCTGC AGAAAGACTT CTGACCGTTC CCCACTACGC CTGCCACTGA 50

CTACACGTCC 60

(2) INFORMATION FOR SEQ ID NO:41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:41:

AGAGTAGGCA CTCTCTAGCA AGCGTCGGAT CAACGCCAGA GCTAAGAATG 50
CACCATCCGC 60

(2) INFORMATION FOR SEQ ID NO:42:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:42:

CAGGCCGAAT GGATGCAGGA AACCTTTGGA CGTGTGCGGA GAGTCGTACC 50

GGGCTGCCTC 60

(2) INFORMATION FOR SEQ ID NO:43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:50 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:43:

AGCCATGACC CAGTAGACTA TGACCGATTC CCCGGAGACC TTCCTGAGCC 50

(2) INFORMATION FOR SEQ ID NO:44:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:40 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single

(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:44:

CAGTAGACTA TGACCGATTC CCCGGAGACC TTCCTGAGCC 40

(2) INFORMATION FOR SEQ ID NO:45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:37 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:45:

GACCCAGTAG ACTATGACCG ATTCCCCGGA GACCTTC 37

(2) INFORMATION FOR SEQ ID NO:46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:31 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:46:

AGTAGACTAT GACCGATTCC CCGGAGACCT T 31

(2) INFORMATION FOR SEQ ID NO:47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:47:

CTGCAGGTCG ACGCTAGC 18

(2) INFORMATION FOR SEQ ID NO:48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:48:

CACGTGGAGC TCGGATCC 18

(2) INFORMATION FOR SEQ ID NO:49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:49:

ACTATAGGGA TCCGAGCTCC ACGTG 25

We claim:

1. A C3b aptamer comprising a nucleic acid molecule including a sequence of nucleotides selected from the group consisting of SEQ ID NOS:1 - 45, which nucleic acid molecule is capable of binding complement protein C3b.
2. The C3b aptamer according to Claim 1, wherein at least one internucleotide linkage is modified to be resistant to nuclease cleavage.
3. The C3b aptamer according to Claim 1, in which at least one internucleotide linkage is a phosphorothioate linkage.
4. The C3b aptamer according to Claim 1 which is detectably labeled.
5. A pharmaceutical composition comprising a C3b aptamer according to Claim 1 in a pharmaceutically acceptable carrier.
6. Use of a C3b aptamer according to Claim 1 in the treatment of a C3b-mediated disease or disorder.
7. A method for detecting C3b *in vivo* in a person comprising the steps of administering to said person a C3b aptamer according to Claim 4 and detecting said C3b aptamer.
8. A method for detecting C3b in a sample *in vitro* comprising the steps of adding to said sample a C3b aptamer according to Claim 4 and detecting the binding of said aptamer to C3b.
9. A method of making an aptamer that binds human complement protein C3b comprising: synthesizing a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1 - 45.

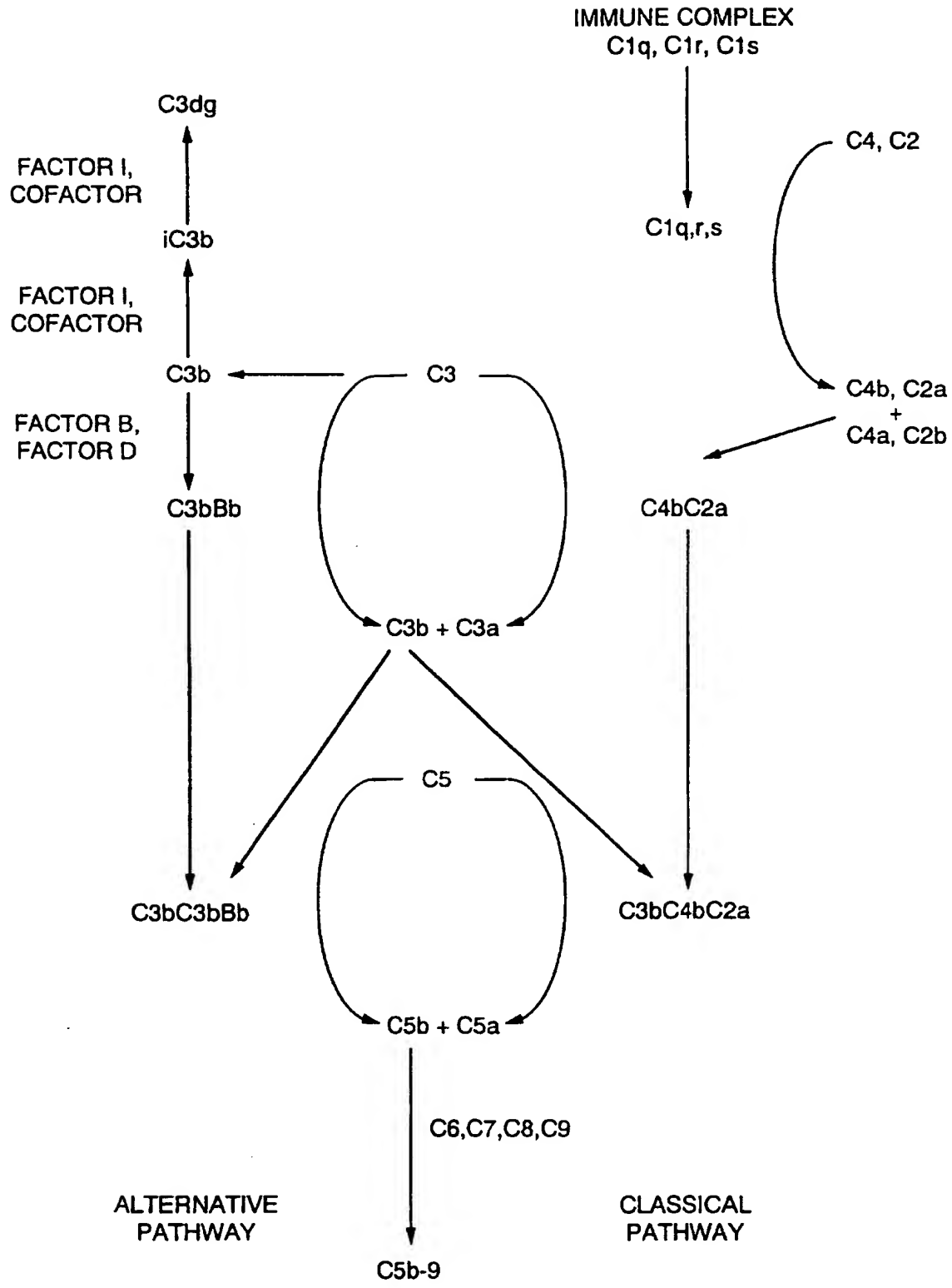
IMMUNE COMPLEX
C1q, C1r, C1s
C1q,r,s
C4, C2
C4b, C2a
+
C4a, C2b
C4bC2a
C3dg
FACTOR I,
COFACTOR
iC3b
FACTOR I,
COFACTOR
C3b
C3
FACTOR B,
FACTOR D
C3bBb
C3b + C3a
C5
C3bC3bBb
C3bC4bC2a
C5b + C5a
C6,C7,C8,C9
ALTERNATIVE
PATHWAY
CLASSICAL
PATHWAY
C5b-9

FIG. 1

```
FAMILY I          A GC---CCA-G GACT-----C TGCTGATGAC TTAGATACCC TCCCGGTCCT GTGGCGGAGG ACC-ACCCC    1*
                  A GC---CCA-G GACT-----C TGCTGATGAC TTAGATACC- TCCCGGTCCT GTGGCGGAGG ACC-AGCCCC   2
                  A GC---CCA-- CACT-----C TGCGA-TGAC TTAGATACC- TCCCGGTCCT ATGGCGGAGG ACCCACCCCC   3
                  A GC---CCA-G GACTATAGCT TGATGATGAC TTAGAT-CCC T-CCGGTCCT GTGGCGGAGG AC--ACC      4

CAGGCA-GAAGCA-GA AGAC-TTTGA CC---TGACT GACTA-AGCT CAGCACTGGC TTAGGTACCC C  5                    FAMILY II
 AGCCA TGACCCA-GT AGAC-TATGA CC-GATTCCC C-GGAGACCT TCCTGA-GCC CTGAACTCCC    6
 AGCCA TGACCCA-GT AGAC-TATGA CC-GATTCCC CCGGAGACCT TCCTGA-GCC -TGAACTCCC    7

ACAG-CA AGACGC--GT TGAC-TAAGA C-TT-TTGAG CAAATG---C T-GCCAGT-- ---AACAACG CC--GGTTCC     8        FAMILY III
ACAG-CA AGACGCA-GT TGAC-TAAGA C-TT-TTGAG CAAATG---C T-GCCAGT-- ---AACAACG CC--GGTCC      9
ACAG-CA AGACGCA-CT TGAC-TAAGA C-TT-TTGAG CAAATG---C T-GCCAGT-- ---AACAACG CC--GGTCC      10
CCAG-CA AGACGCA-GT TGAC-TAAGA C-TT-TTGAG CAAATG---C T-GCCAGT-- ---AACAACG CC--GGTCC      11
ACAG-CA AGAAGGCAGT TGAC-TAAGA C-TT-TTGAG CAAATG---C T-GCCAGT-- ---AACAACG CC--GGAC       12
ACAG-CA AGACGCA-GT TGAC-TAAGA CTTT-TTGAG CACCGG---C T-GCCAGT-- ---AACAACG CC--GGTCC      13
    CA AGACGCA-GT TGAC-TATGA ACCC-CT-A- CACCGG---C T-GGAAGA-- ---CGTAATG CCTGGGTACC CC  14

AG-CG AGACACA-GA TGAC-TTAGA ACCCACAACG CGACTGTTAC TCGCCATA-- T--G-CCGTG TCC  15             FAMILY IV
CAG-CG AGACACA-GA TGAC-TTAGA ACCCACAACG CGACTGT-AC TCGCCATA-- T--GACCGT- TCC  16
CAG-CA -GACACA-GA TGAC-TTAGA ACCCCACAACGCGACTGT-AC TCGCCATA-- T--GCACGT- TCC  17
AGCAG-CA GGTCGCA-GA TGAC-TTAGA ACTAACTTAA ACACTCGT-T TAGCTAGAGC G--GATCC      18

FAMILY V          A -CACT--CAG TGT-CC--GG GGCTCGGGAA AGCAAGGACT -TCAGCTACG CG-GCCGCAG TCTGCCC 19
                  A -CACT--CAG TGT-CC--GG GGCTCGGGAA AGCAAGGACT -TCAGCTACG CGCGACGCAG TCTGCC  20
                  A -CACT--CAG TGT-CC--GG GGCTCGGGAA AGCAAGGACT -TCAGCTACG CCCGCCGCAG TCTGCC  21
                  A -CACT--CAG TGT-CCG-GG GGCTCGGGAA AGCAAGGACT -TCACGTACG CG-GCCGCAG TCTGCC  22
```

* SEQUENCE ID NUMBERS

FIG. 2A

FAMILY VI	ACACCAGA - GGGT - TATG - CAGTT - GAC TG - - CCG - TC - GCCATTGTT - - CTAGG - GA - CGCCTTCGC - GTGC	23*
	ACACCAGA - GGGT - TATG CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGG - GC - GGCCTTCGG C - - TC	24
	ACACCAGA - GGGT - TATG CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGG - - A - CGCCTTCGG - GCCTC	25
	ACACCAGA - - GGT - TATG CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - GA - CTTCGG CAGTC	26
	ACACCAGA - - GGT - TATG CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - CG - CTTCGG CGCTC	27
	ACACCAGA - - GGT - TATG CACTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - CG - CTTCGG CGCTC	28
	ACACCAGA - - GGT - TATG CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - TG - CTTCGG CGCTC	29
	ACACCAGA - - GGT - TATC CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - TCTAGGACC - CG - CTTCG - CGCCTC	30
	ACACCAGA - - GGT - TATG CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - CG - CTTCGG C - CTCC	31
	ACACCAGA - - GGTTTATG CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - CG - CTTCGG C - CTC	32
	ACACCAGA - - GGT - TATG CAGTT - - GAC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - CGGCTTCGG C - CTC	33
	ACACCAGA - - GGT - TATG CAGTT - - - AC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - CGCATTCGG CGCTC	34
	ACACCAGA - - GGT - TATG CAGTT - - - AC TG - - CCG - TC - GCCATTGTT - - CTAGGACC - CG - CTTCGG GATTCC	35
	ACACCAGA - - GGT - TATG CAGTT - - - AC TG - - CCG - TC - GCCATTGTT - - CTAGGACC CCG - CTGCGG GCCTC	36
	ACACCAGA - - GGT - TATG CAGTT - - - AC TG - - CCG - TC GGCCATTGTT - - CTAGGACC - CG - CTTCGG CGCTC	37
FAMILY VII	AGC - TGGG - ACCG CAGTT - - GAA T - - - - - A - TA GACCATAAGG - - CAA - - AAG - CGACTGAAT AAGTTTCCAG CC	38
	AGC - AGGG - ACCG CAGTT - - GAA T - - - - - A - TA GACCATAAGG - - CAA - - AAG - CGACTGAAT AAGTTTCCAG CC	39
FAMILY VIII	A - GC AAGCTGCAGA - AAG - - - A - C TT - CT - - GAC CGT - TC - - CC CACTACGCCT GCCACTGACT. - ACACGTCC -	40
	AGAGTAGGCA CTCTCTA - GC AAGCGTCGGA TCAAC - GCCA GAGCTAAGAA TGCACCA - TC CGC	41
FAMILY IX		
	CAGGCC GAATGGATGC AGGAAACCTT TGGACGTGTG CGG - - AGAGT CGTACCGGGC TGCCTC	42
FAMILY X		

* SEQUENCE ID NUMBERS

FIG. 2B

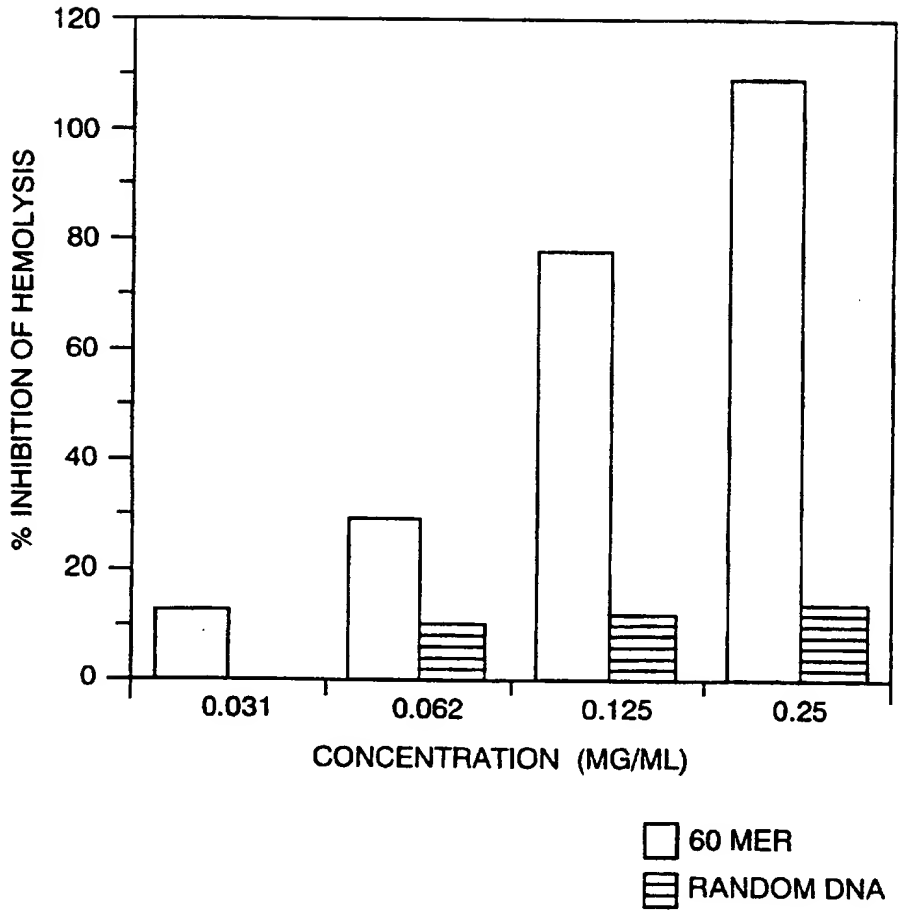
120
100
80
60
40
20
0
% INHIBITION OF HEMOLYSIS
0.031
0.062
0.125
0.25
CONCENTRATION (MG/ML)
60 MER
RANDOM DNA

FIG. 3

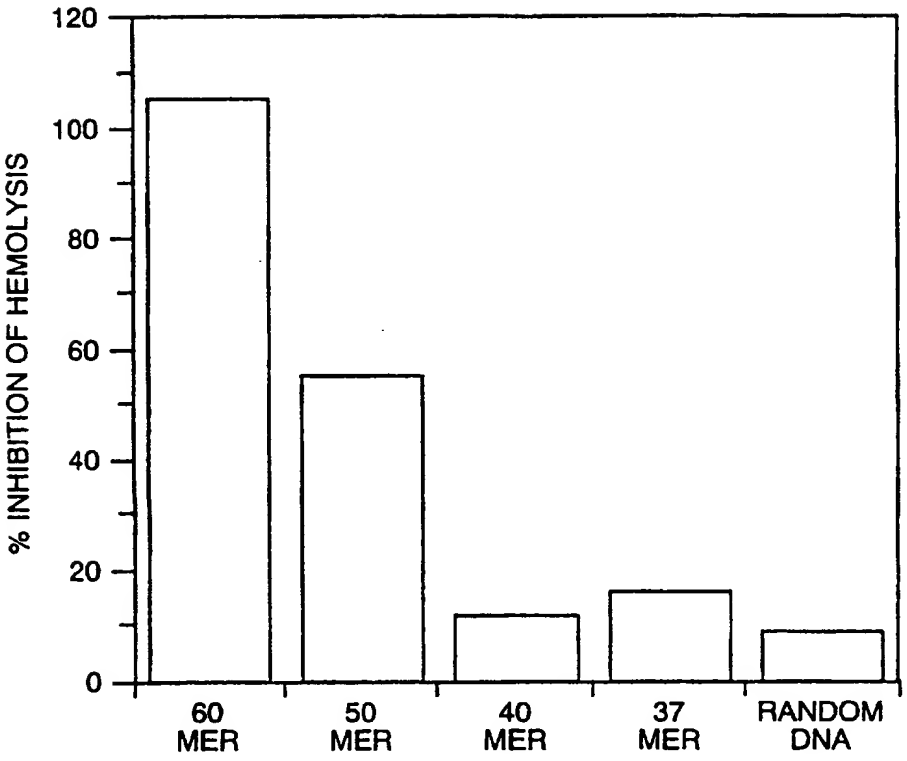
120
100
80
60
40
20
0
% INHIBITION OF HEMOLYSIS
60 MER
50 MER
40 MER
37 MER
RANDOM DNA

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07354

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/11; A61K 31/765
US CL : 536/22.1, 23.1, 25.3; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/22.1, 23.1, 25.3; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE
search terms: C3b, aptamer, phosphorothioate, complement

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRIFFIN et al. In vivo anticoagulant properties of a novel nucleotide-based thrombin inhibitor and demonstration of regional anticoagulation in extracorporeal circuits. BLOOD. 15 June 1993, Vol. 81, pages 3271-3276, see entire document.	1-9
A	BISCHOFBERGER, Norbert et al. Nucleic Acid Targeted Drug Design. New York: Dekker. 1992, pages 579-612, see entire document	1-9
A	US 5,434,257 (MATTEUCCI ET AL) 18 July 1995 (18/07/95), see entire document.	2,3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 AUGUST 1997	Date of mailing of the international search report 05 SEP 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID S. ROMEO Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07354

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07354

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-6 and 9, drawn to a C3b aptamer, a pharmaceutical composition comprising a C3b aptamer, a method of using a C3b aptamer to treat a disease or disorder, and a method of making an aptamer.
Group II, claim(s) 7 and 8, drawn to a methods of detecting C3b *in vivo* and *in vitro*.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the claims are drawn to a product, a method of making said product, and multiple uses of said product. If multiple uses are claimed, the first invention of the category first mentioned in the claims of the application and the first recited use related thereto will be considered as the main invention in the claims, see PCT Article 17(3)(a) and § 1.476(c).